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IMPACT OF ORGANIC LIQUID SEED PRIMING ON GROWTH AND ENZYMATIC METRICS OF WHEAT UNDER VARYING NUTRIENT DOSES

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ABSTRACT

A laboratory experiment was conducted on wheat variety UP-2526 to evaluate the impact of herbal *kunapajala* concentration on growth, chlorophyll and enzymatic metrics under different nutrient concentrations. The design used for experimentation was randomized block design (RBD) with a total of 14 treatments. Among these treatments, hydropriming led to the fastest germination speed, while it also resulted in the shortest mean germination time and the fewest days to 50% emergence, followed by 25% *kunapajala* priming. Seedling development, measured by shoot and root length, seedling length, dry weight of seedlings, and both seedling vigour index-I and seedling vigour index-II, was significantly better with 10% *kunapajala* primed seeds. Additionally, seeds primed with 25% kunapajala showed the highest imbibition rate and α-amylase activity, whereas 10% kunapajala primed seeds exhibited the greatest seed metabolic efficiency among all priming treatments. The higher chlorophyll a content was observed in T_7, T_2, T_3 and T_1 (14.47, 14.17, 14.17 and 12.57 mg/g DW, respectively) as compared to other treatments but was at par with each other. Therefore, seed priming with either 10% or 25% herbal *kunapajala* is highly effective in enhancing germination, seedling growth, biochemical parameters along with chlorophyll content of wheat seeds.

Keywords: α-amylase, chlorophyll, kunapajala, seedling vigour, seed priming,

Introduction

In an era characterized by climate change and increasing temperature variability, the processes of seed germination and establishment are facing significant challenges. The shifting climate conditions, including more extreme temperature fluctuations and altered precipitation patterns, can adversely affect the conditions necessary for seeds to germinate and establish themselves (Sarma *et al.*, 2024a; Sarma & Paul, 2024). To counter these problems, seed priming is a viable option.Seed priming is a pre-sowing method that involves controlled hydration of seeds to kickstart the metabolic processes required for germination, without allowing the radicle to emerge. This approach is commonly used to boost seed performance, ensure uniform germination, and enhance seedling vigour (Sarma *et al.*, 2024b; Sarma & Dutta, 2024). It has the potential to improve seedling vigour and germination rates of fresh seeds, as well as effectively rejuvenate partially aged seeds, thereby enhancing field emergence (Thejeshwini *et al.*, 2019). Seedlings with epigeal germination feature a prominently elongated hypocotyl, which results in a well-developed shoot axis above the soil surface. In contrast, seedlings with hypogeal germination develop a well-formed epicotyl, which remains below the soil surface (Sarma, 2024). Hydropriming is a long-established method for enhancing seed Vigor. Traditionally, it has been employed to accelerate and synchronize seed emergence, as well as to boost the final plant stand. Currently, there is growing interest among researchers in developing environmentally friendly techniques for seed enhancement that promote better germination and more consistent crop establishment. In the field of *vrikshayurveda*, the use of herbal-based *kunapajala* is practiced to improve both the biological efficiency of crops and the health of the soil. *Kunapajala*, also known as *kunapambu*, is a type of liquid manure derived from the Sanskrit term '*kunapa*,' which translates to "resembling the smell of a decaying body." This fermented liquid manure is made from a combination of flesh, animal urine, and marrow (Kumar *et al*., 2020). Today, herbal-based *kunapajala* is utilized to enrich seeds, crops, and soil. It is highly effective as both a soil and foliar application, supporting plant nourishment at various growth stages (Kavya *et al*., 2020; Sudhakar *et al*., 2010). Additionally, it serves as a seed priming technique due to its growth-promoting, nutritional, and antimicrobial properties.

Additional information about herbal *kunapajala*, a type of fermented liquid organic manure, can be found in historical texts such as the *Vrikshayurveda* by Surapala, dated around 1000 AD, and *Lokopakara* by Chavundaraya, written around 1025 AD (Chakraborty *et al*., 2019). Herbal *kunapajala* serves as an effective alternative to chemical fertilizers, offering a valuable nutrient source for crop growth and development. It can be applied to crops at any growth stage. The efficacy of herbal *kunapajala* is attributed to the fermentation process, which breaks down complex compounds into simpler, lower molecular weight molecules, thereby making nutrients more readily available for plant uptake (Neff *et al*., 2003). This liquid organic manure is rich in essential macro and micronutrients (Ankad *et al*., 2017), vitamins, growth hormones such as IAA and GA_3, essential amino acids (Sudhakar *et al*., 2010), and beneficial microbes including nitrogen-fixing symbiotic and free-living bacteria, as well as phosphorus-solubilizing bacteria (Chakraborty *et al*., 2019). Consequently, *kunapajala* stands out as a promising and environmentally friendly plant stimulant, contributing to sustainable crop production and maintaining a safe agro-ecosystem (Kavya and Ushakumari, 2020). Therefore, the current study aims to assess the effects of various doses of herbal *kunapajala*, a liquid organic manure, on Growth, Chlorophyll and Enzymatic metrics of wheat.

Materials and Methods

A field experiment was conducted at the Norman E. Borlaug Crop Research Centre, G.B. Pant University of Agriculture and Technology, Udham Singh Nagar, Uttarakhand, India, during the 2020–21 *rabi* season. The experimental site is located 243.83 meters above msl. at a latitude of 29°01' N and a longitude of 79°48' E. Throughout the experiment, the average weekly maximum temperature ranged from 16.6°C to 37.5°C, with a mean of 27.1°C, while the minimum temperature ranged from 6.1°C to 17.8°C, with an average of 11.9°C. The maximum relative humidity was 83%, fluctuating between 52% and 97%, whereas the minimum relative humidity was 43%. During the crop period, a total of 42.5 mm of rainfall was recorded over 4 rainy days. The experiment was conducted in three replications using a randomized block design (RBD) (Rangaswamy, 1995). Experimental variety of wheat was UP-2526, at a seed rate of 125 kg/ha, sown to a depth of 5 cm with a row spacing of 20 cm using a seed drill. The study included a total of 14 different treatments which were as follows:

Table 1 : Treatment combinations used in the experiment

Treatment Combinations	
T_1	No seed priming + 100% RDN
T_2	Hydropriming + 100% RDN
T_3	10% *kunapajala* priming + 100% RDN+ foliar application of 10% herbal *kunapajala*
T_4	10% *kunapajala* priming + 75% RDN+ foliar application of 10% herbal *kunapajala*
T_5	10% *kunapajala* priming + 50% RDN+ foliar application of 10% herbal *kunapajala*
T_6	10% *kunapajala* priming + no Fertilizer+ foliar application of 10% herbal *kunapajala*
T_7	25% *kunapajala* priming + 100% RDN+ foliar application of 10% herbal *kunapajala*
T_8	25% *kunapajala* priming + 75% RDN+ foliar application of 10% herbal *kunapajala*
T_9	25% *kunapajala* priming + 50% RDN+ foliar application of 10% herbal *kunapajala*
T_{10}	25% *kunapajala* priming + no fertilizer+ foliar application of 10% herbal *kunapajala*
T_{11}	50% *kunapajala* priming + 100% RDN+ foliar application of 10% herbal *kunapajala*
T_{12}	50% *kunapajala* priming + 75% RDN+ foliar application of 10% herbal *kunapajala*
T_{13}	50% *kunapajala* priming + 50% RDN+ foliar application of 10% herbal *kunapajala*
T_{14}	50% *kunapajala* priming + no fertilizer+ foliar application of 10% herbal *kunapajala*

Tn = Treatment number; RDN= Recommended dose of nitrogen

Foliar applications of 10% herbal *kunapajala* were performed at 25, 45, 65, 85, and 105 days after sowing (DAS), excluding treatments T_1 and T_2. The recommended nutrient dose (RDN) was 120-60-30 kg/ha of N-P_2O_5-K_2O. Seeds were treated with various concentrations of herbal *kunapajala* (10%, 25%, and 50%) as well as hydropriming with tap water for 16 hours at a ratio of 1:2 (seed: priming media) (w/v), followed by shade drying to achieve the initial moisture content of 11.6%. To prepare an optimal seedbed for germination, tillage operations were carried out using a tractor-mounted disc harrow and rotavator. The field was then leveled with a pata, and bunds were created around the edges using small bund makers, with additional bunds constructed between the plots by hand.

The standard germination test was conducted following the procedure outlined by ISTA (2004). For this test, fifty wheat seeds were placed on two layers of moist germination paper, which were then covered with another layer of moist paper. The stack was carefully rolled and secured with a rubber band. The rolled paper samples were incubated at a temperature of 20±2°C. Various physiological parameters were assessed, including germination percentage (Kala and Eswari 2019) on the 8th day, speed of germination (Maguire 1962), mean germination time (Ellis and Robert 1981), days to 50% germination (Farooq *et al.* 2019), root length, shoot length, dry weight of seedlings, seedling vigour index-I (SVI-I) and seedling vigour index-II (SVI-II) (Abdul-Baki and Anderson 1973), seed metabolic efficiency (Sikder *et al.* 2009) on the 4th and 8th days post-incubation, and biochemical parameters such as α-amylase activity (Mazumdar and Majumder 2017). The rate of water imbibition was determined using a modified method by Tian *et al.* (2014). The experimental data from the laboratory tests were analyzed using OPSTAT software for a Completely Randomized Design (CRD), and the critical difference was calculated at a 5% significance level.

Results and Discussion

In the current experiment, seeds treated with hydropriming and 25% *kunapajala* priming together achieved the highest germination percentage (99.7%), which was statistically similar to the 10% *kunapajala* priming (99.3%). The highest speed of germination (36.2 seedlings/day) was observed with hydropriming, followed by 25% *kunapajala* priming (32.4 seedlings/day) and 10% *kunapajala* priming (31.9 seedlings/day), which were comparable to each other. The lowest speed of germination was recorded in the no priming treatment (27.4 seedlings/day), which was similar to the 50% *kunapajala* priming treatment (27.6 seedlings/day). Hydropriming also resulted in significantly lower mean germination time and days to 50% emergence (1.57 days and 1.14 days, respectively) compared to other priming treatments, with 25% and 10% *kunapajala* priming following closely behind. The longest mean germination time and days to 50% emergence were observed in the no priming treatment (1.92 days and 1.43 days, respectively), which were comparable to those of the 50% *kunapajala* priming treatment (1.92 days and 1.41 days, respectively).

The longest shoot length was recorded with 10% *kunapajala* priming (12.5 cm), which was comparable to hydropriming (11.8 cm) and 25% *kunapajala* priming (11.5 cm). Among all treatments, the greatest root and seedling lengths were achieved with 10% *kunapajala* priming (10.9 cm and 23.5 cm, respectively), followed by hydropriming (9.72 cm and 21.5 cm) and 25% *kunapajala* priming (9.37 cm and 20.7 cm), which were statistically similar. The highest dry weight of seedlings and seedling vigor index-II (23.0 mg and 2281, respectively) were achieved with 10% kunapajala priming, which were similar to the values obtained with 25% *kunapajala* priming (22.6 mg and 2256, respectively). The maximum seedling vigour index-I (2334) was observed with 10% *kunapajala* priming among all treatments.

The rapid accumulation of germination metabolites and metabolic repair processes facilitated by seed priming enhance the swift translocation and availability of food reserves to developing seedlings (Farooq *et al*., 2019). Consequently, the germination of primed seeds occurs in a shorter time frame. The observed improvement in seedling growth parameters with 10% *kunapajala* priming is likely due to the prompt activation of enzymatic activities and the breakdown of stored nutrients. Seeds treated with 2% *panchagavya*, which includes *Jatropha curcas* and *Pongamia pinnata*, demonstrated higher germination percentages, likely attributed to the activity of beneficial microbes and growth regulators such as GA_3 and IAA in the liquid mixture (Srimathi *et al*., 2013; Sarma & Talukdar, 2024). Ankad *et al*. (2017) found that seeds of *kalamegha* and *ashwagandha* treated with 10% *kunapajala* and *Panchagavya* showed significantly better germination compared to untreated seeds, consistent with findings by Kala *et al*. (2019) and Sumangala and Patil (2009). The improved seedling length observed with *kunapajala* priming is likely due to the presence of plant growth-promoting factors produced by the microbes in *kunapajala* (Kavya *et al*., 2020). However, this benefit diminished at higher concentrations of kunapajala (50%).The improvement in seedling growth parameters and seedling vigor through seed priming with liquid organics has been observed by Kala and Eswari (2019), Ankad *et al*. (2017), Srimathi *et al*. (2013).

Among all priming treatments, 25% *kunapajala* priming exhibited the highest water imbibition rate across all soaking durations (8, 12, 16, and 24 hours). The maximum imbibition rate was recorded between 12 and 16 hours, with rates 50.7% and 62.9% higher at 12 hours compared to 8 and 24 hours of soaking, respectively. For 25% *kunapajala* primed seeds, the imbibition rate was 57.9% and 20.6% higher at 12 and 16 hours of soaking compared to hydropriming. These findings are consistent with those reported by Bormashenko *et al*. (2012) and Ling *et al*. (2014). During the initial hours, the imbibition rate was lower compared to later hours, as starch absorbs water more slowly than protein. Solutes resulting from α-amylase activity enhance water movement into the seeds due to the osmotic potential (Baron 1979).

The highest seed metabolic efficiency was observed with 10% *kunapajala* priming, recording values of 2.99 and 0.96 g/g after 4 and 8 days of incubation, respectively. These values were comparable to those achieved with 25% *kunapajala* priming (2.98 and 0.92 g/g, respectively) and were superior to those of other treatments. In contrast, the lowest seed metabolic efficiency values at both intervals were seen in the control treatment (1.92 and 0.44 g/g, respectively), which were similar to those obtained with 50% *kunapajala* priming (2.00 and 0.54 g/g, respectively). This observation aligns with findings reported by Pal *et al*. (2017). The lower seed metabolic efficiency in seeds treated with 50% *kunapajala* may be attributed to reduced water absorption and decreased levels of GA3 and other enzymes involved in the hydrolytic processes during germination (Marambe *et al*., 1992).

α-amylase activity in primed seeds increased up to 16 hours of soaking but then decreased across all priming treatments. The highest α-amylase activity was recorded in seeds primed with 25% *kunapajala*, showing values of 24.1, 27.1, and 24.6 mg of starch hydrolyzed/g of seeds at 12, 16, and 24 hours of soaking, respectively. These values were statistically similar to those observed with 10% *kunapajala* priming at 12 hours (24.0 mg of starch hydrolyzed/g of seeds) when compared to other treatments. The activity of the *RAmy1A* gene, which is stimulated by transported GA_3, induces α-amylase activity in the endosperm (Kaneko *et al*., 2002). The presence of amino acids, vitamins, and growth regulators (IAA and GA_3) in herbal *kunapajala* (Sudhakar *et al*., 2010) likely promotes α-amylase activity in primed seeds, facilitating the hydrolysis of endosperm starch to provide energy for germination. However, α-amylase activity decreased with higher concentrations of 50% *kunapajala* priming compared to 25% kunapajala priming after 12, 16, and 24 hours of soaking, respectively. This reduction may be due to the negative impact on the synthesis of gibberellic acid, which in turn affects starch hydrolysis.

Table 2 : Impact of various seed invigoration treatments on germination metrics and seedling vigour characteristics in wheat

Treatments	Germination (%)	SOG (Seedling/ day)	MGT (days)	T_{50} (days)	Shoot Length (cm)	Root Length (cm)	Seedling Length (cm)	Seedling dry weight (mg)	SVI-I	SVI-II
No priming	97.7	27.4	1.92	1.43	10.0	8.33	18.9	17.8	1850	1742
Hydropriming	99.7	36.2	1.57	1.14	11.8	9.72	21.5	20.7	2145	2063
10% KJ priming	99.3	31.9	1.75	1.27	12.5	10.9	23.5	23.0	2334	2281
25% KJ priming	99.7	32.4	1.72	1.26	11.5	9.37	20.7	22.6	2062	2256
50% KJ priming	97.3	27.6	1.92	1.41	10.3	8.86	19.2	18.3	1867	1784
SEm (±)	0.6	0.7	0.03	0.03	0.5	0.23	0.5	0.6	46	56
CD (p=0.05)	1.9	2.1	0.09	0.09	1.4	0.74	1.5	1.8	153	180

KJ: *Kunapajala;* SOG: Speed of germination; MGT: Mean germination time; T_{50}: Days taken to 50% germination; SVI-I: Seedling

Table 3 : Impact of various seed invigoration treatments on the rate of water absorption, seed metabolic efficiency, and α-amylase activity in wheat.

Treatments	Water imbibition rate (mg/g dry seed/hr)				Seed metabolic efficiency (g/g)		α-amylase activity (mg of starch hydrolyzed/g of seeds)		
	8 hours	12 hours	16 hours	24 hours	4th DAI	8th DAI	12 hours	16 hours	24 hours
No priming	2.01	14.2	12.1	3.4	1.92	0.44	21.5	21.5	21.5
Hydropriming	3.20	17.6	16.5	6.1	2.80	0.64	23.2	24.9	23.1
10% KJ priming	8.23	23.4	16.5	7.9	2.99	0.96	24.0	25.2	23.0
25% KJ priming	13.70	27.8	19.9	10.3	2.98	0.92	24.1	27.1	24.6
50% KJ priming	1.24	12.5	11.6	1.8	2.00	0.54	19.8	21.3	20.7
SEm (±)	0.96	1.2	0.9	0.3	0.07	0.04	0.6	0.6	0.3
CD (p=0.05)	3.06	3.9	2.7	1.1	0.21	0.11	1.9	1.9	1.1

KJ: *Kunapajala, DAI*: Day after incubation

Table 4: Content of chlorophylls a and b in leaves of wheat

Treatments	Chlorophyll *a* (mg/g DW)	Chlorophyll *b* (mg/g DW)
T_1	12.57	4.43
T_2	14.17	4.46
T_3	14.17	4.50
T_4	11.43	4.19
T_5	11.87	4.42
T_6	9.93	3.53
T_7	14.47	4.50
T_8	11.27	4.35
T_9	11.03	4.29
T_{10}	10.40	3.55
T_{11}	11.30	4.30
T_{12}	11.97	4.41
T_{13}	11.40	4.48
T_{14}	9.43	3.66
SEm (±)	0.52	0.18
C.D.	1.53	0.51

Chlorophyll content is a useful indicator of photosynthetic activity, plant stress, and overall health. Each chlorophyll molecule contains four nitrogen atoms, contributing to the nitrogen content in leaves. The higher levels of total chlorophyll, carotenoids, and both chlorophyll a and b in mustard plants are likely due to the increased nitrogen content from nettle-based herbal *kunapajala*. This herbal preparation, applied both topically and at the root zone, may enhance the availability of nutrients to the plants.

Among the treatments, there was a significant variation in chlorophyll content. The highest levels of chlorophyll a were observed in T_7, T_2, T_3, and T_1, with values of 14.47, 14.17, 14.17, and 12.57 mg/g dry weight, respectively, and these were comparable to each other. In contrast, the lowest chlorophyll a content was found in T_{14}, T_6, and T_{10}, with values of 9.43, 9.93, and 10.40 mg/g dry weight, respectively. For chlorophyll b, higher content was recorded in T_7, T_2, T_3, and T_1, with values of 4.50, 4.50, 4.46, and 4.43 mg/g dry weight, respectively.

Herbal *kunapajala*, when applied topically, is directly absorbed through the leaf's stomata. The chlorophyll content per unit leaf area is also notably affected by the size of the leaf. Moreover, *kunapajala* treatment resulted in the highest leaf area index, and levels of total chlorophyll, carotenoids, and xanthophylls compared to other treatments. Similarly, 3% *panchagavya* spray led to higher pigment content (chlorophyll a and b) in tomatoes during seedling, flowering, and fruiting stages, compared to control and various concentrations of *panchagavya*. Plants treated with *panchagavya* had larger leaves and denser canopies, and similar results were found in *vigna radiata*, *vigna mungo*, *oryza sativa* (Tharmaraj, 2011), and *arachis hypogaea* (Subramaniyan, 2005). Treatments involving 10% herbal *kunapajala* seed priming and foliar spraying with 100% recommended dose of nutrients (RDN) demonstrated the highest chlorophyll levels across all measured parameters. This is likely due to the optimal application of herbal *kunapajala*, which provides a rich supply of macro- and micronutrients, particularly nitrogen, that are readily available to the plants. Herbal-based *kunapajala* is utilized to nourish seeds, crops, and soil effectively. It is particularly beneficial in supporting plants throughout various growth stages and serves as an effective seed priming method (Devi *et al*., 2023b). Research indicates that seed priming with either 10% or 25% herbal *kunapajala* is an eco-friendly approach that enhances seedling emergence, development, and biochemical activity in wheat compared to hydropriming and no priming (Devi *et al*., 2022).

Conclusion

Based on the findings, it can be concluded that seed priming with either 10% or 25% herbal-based *kunapajala* is an environmentally friendly technique for enhancing wheat seed emergence, seedling growth, and biochemical activity and chlorophyll content compared to hydropriming and no priming treatments.

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